I.R., Wrana, J.L., and Dennis, J.W. (2004). Science *306*, 120–124.

Penninger, J.M., Irie-Sasaki, J., Sasaki, T., and Oliveira-dos-Santos, A.J. (2001). Nat. Immunol. *2*, 389–396.

Sospedra, M., and Martin, R. (2005). Annu. Rev. Immunol. *23*, 683–747.

Starossom, S.C., Mascanfroni, I.D., Imitola, J., Raddassi, K., Hernandez, S.F., Bassil, R., Croci, D.O., Cerliani, J.P., Wang, Y., Elyaman, W., et al. (2012). Immunity *37*, this issue, 249–263.

Trapp, B.D., and Nave, K.A. (2008). Annu. Rev. Neurosci. *31*, 247–269.

# Regulate Globally, Act Locally: Adrenergic Nerves Promote Leukocyte Recruitment

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**In this issue of *Immunity*, Scheiermann et al. (2012) demonstrate that circadian regulation of the expression of endothelial cell adhesion molecules via adrenergic innervation of local vasculature promotes clinically significant changes in leukocyte homing and bone marrow engraftment.**

One of the most beneficial aspects of the immune response is the spatial precision with which it is focused. Even the innate immune response (with the exception of septic shock and systemic inflammatory response syndrome) is generally exquisitely localized. Indeed, even as I type this draft with an ingrown fingernail, all the tumor, rubor, calor, and dolor is confined to the left side of the nail bed of the affected finger. Degranulated dermal mast cells, chemokine-secreting macrophages, and activated endothelial cells in the affected vascular bed are recruiting neutrophils to kill bacteria and start the process of wound repair. Local circulation on the other side of my finger goes on blissfully unaware. Direct observations of leukocyte emigration at sites of inflammation in vivo reveal that, even within the inflamed tissue, leukocytes preferentially emigrate from a restricted set of postcapillary venules. It is a good thing for us as a species that control of inflammation is so local—if neutrophils were pouring into my alveoli as fast as they were entering the tip of my finger, I'd be deathly ill from pneumonia before I finished the next paragraph.

Research on the inflammatory response has focused on mechanisms regulating local control. With the discovery of inflammation-induced adhesion and signaling molecules on endothelial cells of postcapillary venules, glycosaminoglycan-binding diffusible activators of inflammation such as chemokines designed to act locally, and short-lived proinflammatory molecules such as prostaglandins, nitric oxide, and reactive oxygen intermediates, the molecular regulation of the inflammatory response could be explained at the cellular level.

The idea that the CNS can regulate inflammation—i.e., that emotional stress can exacerbate inflammatory bowel disease, arthritis, etcetera, whereas a positive mental attitude gets one over physical infirmities—has been around for a long time. However, the scientific basis of this phenomenon has come to light relatively recently. Lymphocytes, myeloid cells, and endothelial cells bear receptors for and respond to growth factors, cytokines, and neurotransmitters that were originally thought to be specific for the nervous system (Wong et al., 2002). There is good evidence for bidirectional communication between the nervous system and the immune system (Andersson and Tracey, 2012).

The sympathetic nervous system (more properly, adrenergic stimulation) has been shown to play a major role in regulating the inflammatory response. Adrenergic neurotransmitters and hormones (acting through adrenergic β2 receptors) are traditionally thought of as anti-inflammatory, which fits with their stimulation by the response-to-injury physiology of the "fight-or-flight" response. However, proinflammatory actions of adrenergic neurons have been described (Arima et al., 2012).

The parasympathetic nervous system regulates a "cholinergic anti-inflammatory pathway" (Andersson and Tracey, 2012) that is particularly potent in limiting the continued secretion of cytokines such as tumor necrosis factor alpha (TNF-α) by peripheral tissues. Macrophages have nicotinic receptors for the neurotransmitter acetylcholine, which is secreted in inflamed tissue by branches of the vagus nerve. Upon binding acetylcholine, these receptors transduce a signaling pathway that inhibits NF-κB activation and dampens the proinflammatory signals from toll-like receptors and cytokines (Andersson and Tracey, 2012). The anti-inflammatory effects of parasympathetic stimulation are pathophysiologically relevant. Stimulation of this pathway can protect rodents from the proinflammatory mediators released in response to hemorrhagic shock (Luyer et al., 2005). These effects can be ablated by vagotomy or pharmacologic blockade of the receptors.

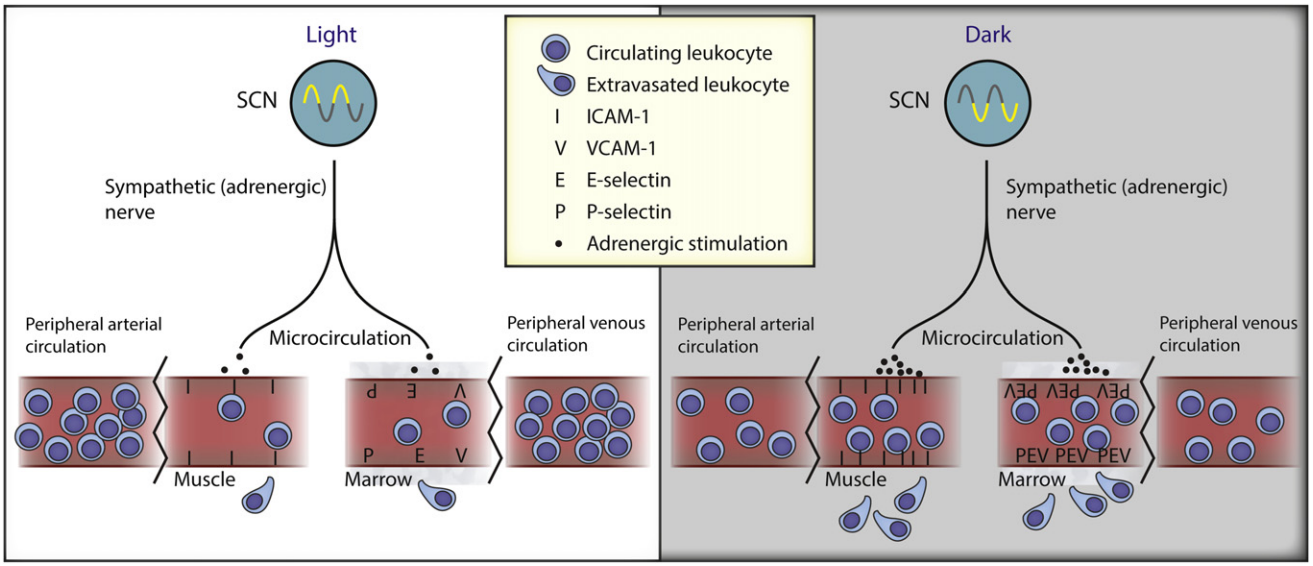


**Figure 1. Adrenergic Innervation Transmits Circadian Variation in Leukocyte Adhesion Molecule Expression**
Light input from the retina is organized with other circadian signals in the brain by the suprachiasmatic nucleus of the hypothalamus (SCN). Sympathetic (adrenergic) nerve activity innervating the microvasculature of the body varies cyclically as well. In mice, low-level tonic innervation during the daytime induces basal levels of endothelial cell adhesion molecules (ICAM-1, VCAM-1, and P- and E-selectin) to support basal levels of leukocyte rolling, adhesion, and extravasation under homeostatic conditions and lower inducible levels under inflammatory conditions. In the absence of inflammation, with low levels of leukocyte homing to the tissues, circulating blood leukocyte levels are at their peak. Shortly after the onset of darkness, increased sympathetic activity to the same vascular beds induces site-selective upregulation of ICAM-1 in muscle; VCAM-1 and E- and P-selectin in bone marrow sinusoids. This results in increased leukocyte homing and extravasation under homeostatic conditions and higher inducible levels of extravasation under inflammatory conditions. In the absence of inflammation, the increased homeostatic extravasation results in lower circulating blood leukocyte levels.

But there is an even higher and less direct level of control than this. Circadian rhythm, which cyclically regulates many physiologic functions with a daily periodicity, is entrained by the light cycle and controlled by clock genes in the suprachiasmatic nucleus of the hypothalamus that regulate the periodic expression of transcription factors through a series of positive and negative feedback loops. Body temperature, levels of cortisol and of a variety of other hormones and cytokines, blood pressure, soluble TNF receptor levels, and circulating leukocyte counts have long been known to exhibit diurnal variations. Frenette and colleagues previously showed that circadian oscillation of secretion of CXCL12 by bone marrow stromal cells in mice was governed by adrenergic stimulation from sympathetic nerves innervating bone marrow (Méndez-Ferrer et al., 2008). Stimulation led to inhibition of CXCL12 synthesis, resulting in peak release (perhaps more correctly, decreased retention) of hematopoietic stem cells into circulation 5 hr after the onset of light and declining to a minimum 5 hr after the onset of darkness, for mice on a 12 hr light cycle (Méndez-Ferrer et al., 2008).

The important question is whether these diurnal variations are of clinical significance. There is extensive epidemiologic evidence that they could be. Inflammatory and immune diseases show a diurnal pattern of onset and exacerbation (Maury et al., 2010). Analysis of 3,000 patients with myocardial infarction revealed a significant increase in onset in the morning, with a 3-fold difference between the incidence of onset at 9 a.m. versus 11 p.m. (Muller et al., 1985). Of interest, this circadian temporal pattern was not seen in patients already taking beta (adrenergic) blockers therapeutically.

In this issue of *Immunity*, Scheiermann et al. (2012) demonstrate that circadian regulation of the inflammatory response, mediated via sympathetic nerve innervation of local vasculature, is significant enough to have implications for the outcome of the inflammatory response. In mice on a strict light-dark cycle, physiologic (homeostatic) homing of leukocytes to bone marrow and monocytes to skin exhibits a diurnal variation that peaks 1 hr after lights go off (Figure 1). This corresponds to the nadir in circulating blood leukocytes. They further show that this homing is due to corresponding increases in the expression of the appropriate adhesion molecules on the endothelial cells. These circadian differences can be abolished by interruption of sympathetic innervation of the local vasculature or knockout of the appropriate adrenergic receptor on the endothelium; they can be exaggerated in wild-type mice by the administration of adrenergic agonists. Furthermore, they are ablated in mice lacking *Bmal1*, one of the transcription factors that serves as a master regulator of circadian rhythms, and in wild-type mice subjected to "jet lag"—a light cycle in which mice are subjected to a full 24 hr of light. A wealth of data is presented, and although some of the differences are small in magnitude, they are all internally consistent.

The big advance here is in showing that these circadian differences can be clinically significant. Lethally irradiated mice transplanted with limiting numbers of bone marrow cells at night (the peak of homing activity) all survive, whereas half of those that receiving the same number of bone marrow cells in the daytime die. A similar effect was achieved by adding a β3-adrenergic agonist to the regimen.

Finally, in models of sickle cell crisis and septic shock wherein leukocyte adhesion to endothelium contributes to the pathology and ultimate demise of the mice, survival was significantly worse if inflammation was induced at night rather than during the day.

One fascinating and unresolved question relates to the local regulation of this global rhythm: How do the same adrenergic stimulus and receptors induce different adhesion molecules when acting on different vascular beds? Intercellular cell adhesion molecule-1 (ICAM-1) is upregulated in venules of the cremaster muscle, where there were circadian differences in leukocyte adhesion, but not rolling. E- and P-selectin and vascular cell adhesion molecule-1 (VCAM-1) are upregulated on bone marrow sinusoidal endothelium, where leukocyte rolling exhibited diurnal variation and was the rate-limiting step (Figure 1). Obviously, there is more subtlety to circadian rhythm than just output from sympathetic nerves, and much more to be learned.

An obvious teleologic reason for the nighttime peak of leukocyte homing would be to give the mice the best inflammatory response when they most need it—when they are active—given that the inflammatory response evolved to heal wounds and fight off invading microorganisms (not to protect from iatrogenically injected TNF-α). Mice are nocturnal, whereas humans are active during the day; therefore, it stands to reason that the exact timing of peak peripheral-blood leukocyte levels and peak expression of endothelial ligands for leukocyte adhesion would be the opposite in humans, and indeed they are (Bertouch et al., 1983; Lucas et al., 2008). Of course, in modern society, man is not on a strict on/off light-dark cycle, and many stimuli, in addition to photic cues, can entrain circadian rhythms. Therefore, the degree of circadian entrainment of our inflammatory system would be expected to be much lower than that of the experimentally controlled mice. In that light, it is even more amazing that circadian cycles of circulating leukocyte numbers and myocardial infarcts are seen in outbred human populations. In summary, the fascinating data provided by this study make it clear that circadian fluctuations in the inflammatory response should be considered when designing bone marrow and solid-organ transplant protocols and anti-inflammatory therapies.

## REFERENCES

Andersson, U., and Tracey, K.J. (2012). J. Exp. Med. *209*, 1057–1068.

Arima, Y., Harada, M., Kamimura, D., Park, J.H., Kawano, F., Yull, F.E., Kawamoto, T., Iwakura, Y., Betz, U.A., Márquez, G., et al. (2012). Cell *148*, 447–457.

Bertouch, J.V., Roberts-Thomson, P.J., and Bradley, J. (1983). Br. Med. J. (Clin. Res. Ed.) *286*, 1171–1172.

Lucas, D., Battista, M., Shi, P.A., Isola, L., and Frenette, P.S. (2008). Cell Stem Cell *3*, 364–366.

Luyer, M.D., Greve, J.W.M., Hadfoune, M., Jacobs, J.A., Dejong, C.H., and Buurman, W.A. (2005). J. Exp. Med. *202*, 1023–1029.

Maury, E., Ramsey, K.M., and Bass, J. (2010). Circ. Res. *106*, 447–462.

Méndez-Ferrer, S., Lucas, D., Battista, M., and Frenette, P.S. (2008). Nature *452*, 442–447.

Muller, J.E., Stone, P.H., Turi, Z.G., Rutherford, J.D., Czeisler, C.A., Parker, C., Poole, W.K., Passamani, E., Roberts, R., Robertson, T., et al. (1985). N. Engl. J. Med. *313*, 1315–1322.

Scheiermann, C., Kunisaki, Y., Lucas, D., Chow, A., Jang, J.-E., Zhang, D., Hashimoto, D., Merad, M., and Frenette, P.S. (2012). Immunity *37*, this issue, 290–301.

Wong, K., Park, H.T., Wu, J.Y., and Rao, Y. (2002). Curr. Opin. Genet. Dev. *12*, 583–591.

# Guanylate-Binding Proteins: Niche Recruiters for Antimicrobial Effectors

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**There are fundamental questions regarding how IFN-γ activates host cells to eliminate intracellular pathogens. In this issue of *Immunity*, Yamamoto et al. (2012) demonstrate a critical role for the p65 guanylate-binding proteins (GBPs) in this process during infection with *Toxoplasma gondii*.**

For over 30 years, IFN-γ has been recognized as an important cytokine capable of activating a wide variety of host cells, perhaps most notably macrophages, to kill an array of intracellular bacteria and parasites, including *Toxoplasma gondii*, *Trypanosoma cruzi*, *Leishmania* species, *Mycobacterium* species, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Chlamydia trachomatis* (Taylor et al., 2004). Although the essential role of IFN-γ in immunity to these organisms has long been appreciated, the specific mechanisms by which it confers protection are still being defined. The finding that IFN-γ can induce macrophages to produce toxic oxygen radicals (mediated in mice partly through increased expression